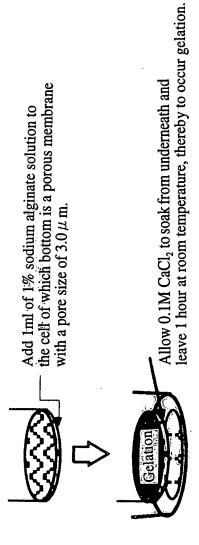
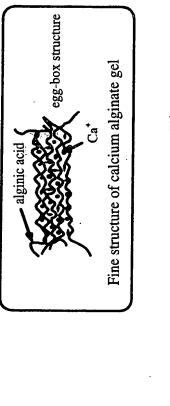
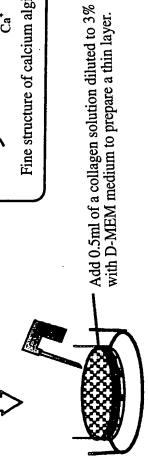


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Gelátion in CO_2 incubator at 37% for 20 minutes.

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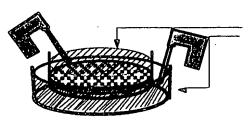


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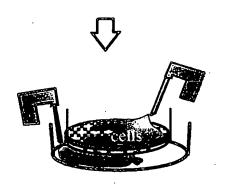
Method of Forming a Structure Having Multiple Cell Layers (as

amended)



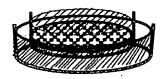
Add D-MEM medium, 2 ml into the cell and 3 ml to a schale, and then infiltrate overnight.

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Remove the medium in the cell or schale, spread 10,000 cells (0.5ml) of fibroblast collected by trypsin treatment, and add 3ml of D-MEM medium to the schale.

Leave in a 0.5% CO₂ incubator for about 1 hour at 37°C to allow the attachment of fibroblast.



Exchange the medium on Day 2 after culture, and further culture for a period of one day to form a confluent cell monolayer.





Laminate structure of gel and cultured cell monolayer



culturted fibroblast monolayer collagen gel

calcium alginate gel membrane filter $(3.0 \,\mu\,\text{m})$



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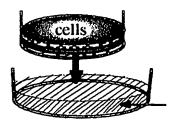
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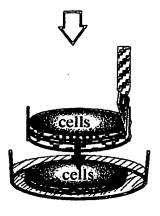
Method of Forming a Structure Having Multiple Cell Layers (as

amended)

FIG. 3

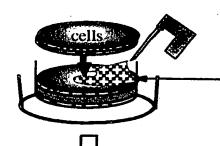


Soak the cell into 0.1M EDTA solution to dissolve the calcium alginate gel and liberate it from the membrane filter



Remove extra water from the cell by suction, insert a scalpel through the inner wall of the cell to hollow the filter, thereby suspending a cell sheet of collagen gel in D-MEM medium.





Add 0.5ml of a collagen solution onto the gel sheet which is not suspended, and overlay a gel formed in the same manner as above



Culture three cell layers laminated with a sandwiched collagen gel, in D-MEM medium.

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